

AMENDMENTS TO THE CLAIMS

The following listing of the claims replaces all prior claim listings.

LISTING OF CLAIMS:

Claim 1 (Original): A method of synthesizing a mammalian, essentially pure, glycosylated Dkk protein comprising the steps of:

- (a) harvesting culture media from a mammalian cell line with a nucleic acid encoding a mammalian Dkk protein in a replicating vector that synthesizes Dkk protein, wherein said Dkk protein comprises a signal peptide for expression and secretion into media of said Dkk protein;
- (b) purifying the filtered culture media across an affinity gradient column;
- (c) collecting Dkk protein containing fractions from the column;
- (d) concentrating the Dkk protein containing fractions in a phosphate buffered saline in the presence of a detergent and EDTA to produce a concentrated, mammalian, essentially pure, glycosylated Dkk protein.

Claim 2 (Original): The method of claim 1, wherein the method further comprises purifying the Dkk protein using preparative and analytical size-exclusion chromatography.

Claim 3 (Original): The method of claim 1, further comprising treating the culture media with one or more protease inhibitors.

Claim 4 (Original): The method of claim 1, further comprising the step of filtering the culture media prior to purifying the culture media.

Claim 5 (Original): The method of claim 1, wherein the detergent is Tween, CHAPS, N-octyl- β -D-glucoside, triton X-100, or Nonidet P40.

Claim 6 (Original): The method of claim 1, wherein the affinity column is a metal affinity column.

Claim 7 (Currently Amended): The method of claim [[1]] 2, wherein the size exclusion column is a Superose-12 column, a Superdex-200 column, a Sephacryl column, or a Sephadex column.

Claim 8 (Original): The method of claim 6, wherein the metal is nickel, zinc, or iron.

Claim 9 (Previously Presented): The method of claim 5, wherein the Tween is Tween-20 in the amount of about 0.01% to about 1% Tween-20, and EDTA is present in the amount of about 0.01 mM to about 2 mM EDTA.

Claim 10 (Previously Presented): The method of claim 5, wherein Tween-20 is present from about 0.005% to about 0.1%, or N-octyl- β -D-glucoside is present from about 0.05 to about 0.7%; and the EDTA is present in the amount of about 0.5 nM EDTA.

Claim 11 (Previously Presented): The method of claim 5, wherein the N-octyl- β -D-glucoside is present in the amount from about 0.05% to about 0.7%, and EDTA is present in the amount of about 0.5 M.

Claim 12 (Original): The method of claim 9, further comprising the step of lyophilizing the essentially purified Dkk protein.

Claim 13 (Original): The method of claim 1, wherein the Dkk protein is Dkk1.

Claim 14 (Original): The method of claim 13, wherein the Dkk1 protein is human Dkk1.

Claim 15 (Original): The method of claim 3, wherein the treating step is additionally performed in the presence of a salt and imidazole.

Claim 16 (Previously Presented): The method of claim 15, wherein the salt is NaCl, LiCl, or KC1, and wherein the salt is in a final concentration of about 100 mM to about 1 M, and the imidazole is present in a final concentration of about 0.5 mM to about 50 mM.

Claim 17 (Original): The method of claim 15, wherein the salt is NaCl and is present at a final concentration of about 500 mM, and the imidazole is present at a final concentration of about 5 mM.

Claim 18 (Original): The method of claim 1, wherein the affinity gradient is an imidazole gradient of about 5 to about 1,500 mM imidazole in a metal column, and wherein the Dkk protein is tagged with histidine.

Claim 19 (Original): The method of claim 18, wherein the imidazole gradient is about a 20 mM to about a 1,000 mM imidazole gradient.

Claim 20 (Original): The method of claim 18, wherein the Dkk protein is human Dkk1, and the metal column is a nickel column.

Claim 21 (Original): The method of claim 1, wherein the mammalian cell line is HEK293T cells or HEK293 EPNA cells.

Claim 22 (Original): A purified, glycosylated Dkk protein produced by the method of claim 1.

Claim 23 (Original): The purified, glycosylated Dkk protein of claim 22, wherein the essentially purified, glycosylated Dkk1 protein is a human Dkk1 protein.

Claim 24 (Original): The purified, glycosylated Dkk protein of claim 22, wherein the Dkk protein further comprises a selectable tag.

Claim 25 (Original): The purified, glycosylated Dkk protein of claim 24, wherein the selectable tag is a c-myc tag or a His₆-tag.

Claim 26 (Original): The purified, glycosylated Dkk protein of claim 25, wherein the selectable tag is a His₆-tag.

Claim 27 (Previously Presented): The purified, glycosylated Dkk protein of claim 25, wherein the Dkk protein further comprises a proteolytic cleavage site between the selectable tag and the Dkk protein.

Claim 28 (Original): A purified, glycosylated, mammalian Dkk1 protein having at least one of the following properties:

- (a) a molecular weight of approximately 40 kD±2.0 kD as determined by SDS-PAGE;
- (b) inhibits Wnt3A activity; and
- (c) co-immunoprecipitates a LRP5 protein or a fragment thereof comprising the ligand binding domain.

Claim 29 (Previously Presented): A purified, glycosylated, mammalian Dkk1 protein having at least one of the following properties:

- (a) a molecular mass of about 36.1 kD to about 36.8 kD as determined by ESI-MS;
- (b) a weight average molar mass of about 36 to about 46 kD of a Dkk1;
- (c) an S-value of 2.8 for monomeric Dkk1 protein as determined by AUC analysis;

- (d) a weight average molecular mass of about 74 kD of a Dkk1 homodimer as determined by SEC-MALLS;
- (e) a Dkk1-LRP5 binding stoichiometry of 1:1 as determined by AUC and/or SEC-MALLS;
- (f) a molar extinction coefficient of $1.96 \times 10^4 \cdot M^{-1} \cdot cm^{-1}$ as determined based on the amino acid sequence of Dkk1 and changes in refractive index of Dkk1 due to glycosylation as measured using a high performance liquid chromatography refractometer;
- (g) a change in specific refractive index increment of Dkk1 due to glycosylation as measured using a differential refractometer; and/or
- (h) a dn/dc value of 0.186 for an unmodified Dkk1 protein and a dn/dc value of 0.130 to 0.180 of glycosylated protein due to post translational modifications.

Claim 30 (Original): The purified protein of claim 29, wherein the Dkk1 protein is human Dkk1 protein.

Claim 31 (Original): The purified protein of claim 29, wherein the protein comprises a His tag.

Claim 32 (Original): The purified, glycosylated Dkk protein of claim 24, wherein the selectable tag is a c-myc tag.